

**GROWTH AND SURVIVAL OF PERCHLORATE-REDUCING BACTERIA IN MEDIA CONTAINING ELEVATED PERCHLORATE CONCENTRATIONS AND UV-C CONDITIONS.** K. F. Bywaters<sup>1</sup>, C. P. McKay<sup>2</sup>, and R. C. Quinn<sup>2</sup>, <sup>1</sup>NASA Postdoctoral Program (Kathryn.f.bywaters@nasa.gov), <sup>2</sup>NASA Ames Research Center, Moffett Field, CA 94035

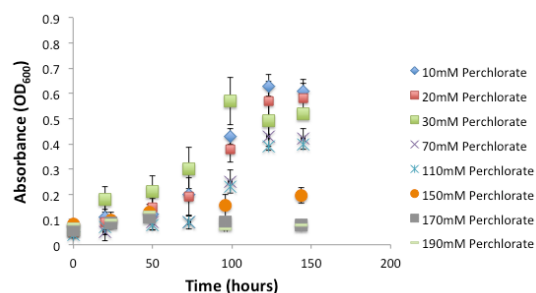
**Introduction:** The identification of perchlorate ( $\text{ClO}_4^-$ ) on Mars [1,2] has led to the possibility that complete redox couples are available for microbial metabolism in contemporary surface environments [3]. Perchlorate-reducing bacteria (PRB) utilize  $\text{ClO}_4^-$  and chlorate ( $\text{ClO}_3^-$ ) as terminal electron acceptors due to the high reduction potential [3]. Additionally,  $\text{ClO}_4^-$  salts have been suggested as a possible source of brines on Mars [4,5] and spectral evidence indicates that the hydration of  $\text{ClO}_4^-$  salts in the regolith of Martian is linked to the surface recurring slope lineae (RSL) [6]. For these reasons PRB may serve as analog organisms for possible life on Mars. However, there is very little information on the viability of PRB in aqueous environments that contain high levels of perchlorate.

Microorganisms on or near the surface of Mars, such as in the RSL, would potentially be exposed to high-salinity and high ultraviolet radiation environments. Under these extreme conditions, microorganisms must possess mechanisms for maintaining continued high genome fidelity. To assess possible microbial viability in contemporary Mars analog environments we are investigating the tolerance of two PRB strains in aqueous conditions under high UV-C conditions and high  $\text{ClO}_4^-$  concentrations.

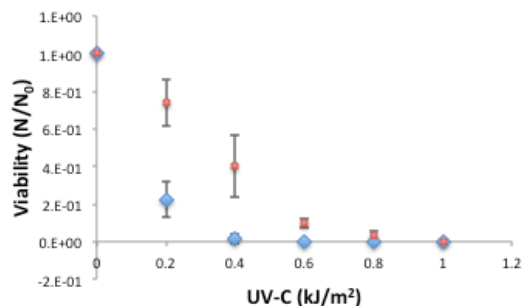
**Methods:** *Salt tolerance:* *Azospira suillum* strain PS was anaerobically grown in media containing: 10, 20, 30, 70, 110, 150 and 170 mM  $\text{ClO}_4^-$ . The protocol was repeated with the culture from the highest concentration that exhibited growth. *UV-C tolerance:* *A. suillum* and high-salinity PRB *Marinobacter multirespiro* were plated and irradiated at 253.7 nm. Enumeration of cultivable microorganisms was evaluated by colony-forming units. Cell viability is expressed as numbers of colonies on plates not exposed to UV-C over the number of colonies on plates exposed to UV-C ( $N/N_0$ ).

**Results and Discussion:** *Salinity tolerance:* The salinity tolerance (Fig. 1) of *A. suillum* was shown to be up to 110 mM  $\text{ClO}_4^-$ , which equates to  $\sim 10.5 \text{ g ClO}_4^- \text{ l}^{-1}$ . In contrast,  $\text{ClO}_4^-$  brines, on Mars, may form with water activities as low as  $\sim 50\%$  [7]. While these results are strain specific, they show that in contrast to other studies which investigated tolerance in NaCl solutions [8], microbial viability may also be limited by high  $\text{ClO}_4^-$  concentrations. *UV-C tolerance:* *A. suillum* grown in 110 mM  $\text{ClO}_4^-$  showed UV-C resistance up to  $0.4 \text{ kJ/m}^2$  and *M. multirespiro* up to  $0.6 \text{ kJ/m}^2$  (Fig. 2). While mineral inclusion and surface material may shield microorganisms to some extent, radiation re-

sistance (including ionizing radiation), is a critical factor related to the possible presence of extant life on Mars.



**Figure 1.** Growth curves for *A. suillum* grown in increasing concentrations of perchlorate.



**Figure 2.** Viability at increasing UV-C for *A. suillum* (♦ Blue) *M. multirespiro* (• Red).

Our results show that while PRB that can tolerate high concentrations of  $\text{ClO}_4^-$ , can also tolerate higher UV-C, potential viability on Mars will be site dependent and limited by local water availability, even in brine environments.

**References:** [1] Hecht et al. (2009) *Science* 325, 64-67 [2] Glavin et al. (2013) *J. Geophys. Res.* 118, 1955-1973 [3] Coates and Achenbach (2004) *Nature Rev. Microbiol.* 2, 569-580 [4] Zorzano et al. (2009) *Geophys. Res. Lett.* 36, 20 [5] Davila et al. (2013) *Int. J. Astrobiol.* 12, 321-325 [6] Ojha et al. (2015) *Nat. Geo.*, 8,829-832 [7] Martin-Torres et al. *Nat. Geo* 8, 357-361 [8] Logan et al (2001). *Wat. Res.* 35, 3034-3038.

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